Linearity App

User Manual

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Introduction

The Linearity App is designed to calculate analyte concentrations in samples using standard titration data from common laboratory assays such as sandwich enzyme-linked immunosorbent assay (ELISA), bicinchoninic assay (BCA), and Griess assay.

The app processes assay data provided in Excel (.xlsx) or CSV (.csv) format. The input file should include:

- Sample descriptions (names and dates)
- Well types (standard, sample, or negative control)
- Sample dilution factors
- Measured optical densities

Using linear regression, the app establishes a mathematical relationship between optical densities and known standard concentrations. This regression is then applied to calculate analyte concentrations in the samples, adjusting for the corresponding dilution factors.

The final results can be exported as an Excel (.xlsx) or CSV (.csv) file and/or saved as an assay protocol in a Word (.docx) file.

How to use the app

The first step in data processing is preparing a file with raw data. To simplify this process, users can utilize the ‘Make Template’ section in the sidebar.

Template Parameters

- **Start:** The initial concentration of the standard (default: 100).
- **Step:** The dilution factor for titration (default: 2). Using this default setting, the app generates a titration curve with the following concentrations: 100, 50, 25, 12.5, 6.25, 3.13, 1.56.
- **Standard columns:** The number of columns in the plate allocated for standard titration (default: 2).
- **N samples:** The number of sample entries to be included in the template file (default: 10).

Generating and Saving the Template

Once the parameters are set, users can generate and download the template file by clicking the ‘Download Template’ button. The file can be saved in either Excel (.xlsx) or CSV (.csv) format, with the preferred format selected via the ‘File type’ widget.

CSV Format Options

The app supports two CSV dialects:

- Comma-separated files (CSV, ",") – Uses a dot (.) as the decimal separator.
- Semicolon-separated files (CSV, ";") – Uses a comma (,) as the decimal separator.

The preferred format can be specified in the ‘Sep’ field.

Importing data

To begin data analysis, open the saved template file in Excel or another spreadsheet program and enter the necessary information.

1. Fill sample names and dates. Enter sample names and dates in the corresponding columns. These should be added in rows where the "type" column is labeled as "sample".
2. Modifying the Type Column if needed. The "type" column can be adjusted as needed; the order of standard, negative control (NC), and sample wells does not affect calculations. Additional wells can be added if necessary, but the "type" column must contain only one of the following values: "standard", "NC", "sample".
3. Fill dilution factors. Enter the dilution factors for each sample in the "dilution" column. Every sample must have a corresponding dilution value.
4. Copy Optical Density (OD) Values. Copy the measured OD values into the "OD" column. Overscale values or excluded wells should be replaced with "NA".
5. Additional columns can be included in the file, but they will not affect concentration calculations. However, they may be useful for data aggregation later
6. Once all modifications are made, save the file before proceeding.

Open the Linearity App window. Click the "Browse" button in the "Import Data" section of the sidebar. The app will validate the file before processing, ensuring the following conditions are met:

- The six specified columns ("type", "sample name", "date", "dilution", "conc", and "OD") are mandatory.
- The "dilution", "conc", and "OD" columns contain only numeric values.
- The "type" column contains only "standard", "NC", or "sample", with no missing values.
- Every sample row has a valid, non-missing, positive dilution factor.
- At least four standard wells are included to ensure reliable standard curve fitting.

If the file passes validation, a data table will appear in the main panel, allowing users to review and analyze the imported data.

Standard Curve Analysis

To proceed with data analysis, navigate to the “Standard Curve” tab. This section provides graphical representations of the standard titration and model fitting accuracy.

The main panel displays two plots:

1. Standard Curve (Left Plot)
   - Shows the fitted regression line applied to the standard titration data.
   - Provides a general visualization of the relationship between optical density (OD) and analyte concentration.
2. Residuals Plot (Right Plot)
   - Displays fitted values vs. residuals, helping assess the accuracy of the model.

- Highlights potential patterns in residuals, which could indicate systematic errors in the regression fit.

The Linearity App supports two types of regression models for describing the relationship between analyte concentrations and optical density values:

1. Linear Regression ($y \sim bx + c$)

Can be fitted using either ordinary least squares method or Deming regression (more precise, as it accounts for measurement errors in both the independent and dependent variables).

2. Quadratic Regression ($y \sim ax^2 + bx + c$)

It is fitted using ordinary least squares method and is more suitable when the titration curve exhibits slight curvature, as it improves the accuracy of modelling.

Regression coefficients (est) and their 95% confidence intervals (ci.lower and ci.upper) are displayed in the table below two plots.

By default, the app applies log-transformed values to both concentrations and optical densities, enhancing linearity in the regression model. However, users can switch to linear scaling by selecting the “Linear Scales” checkbox.

The results table can be exported as an Excel (.xlsx) or CSV (.csv) file by clicking the “Download Results” button.

Result Table

By clicking the "Results" tab, users can view a table displaying calculated analyte concentrations adjusted for the dilution factor. The table includes four mandatory columns—"sample.names", "sample.dates", "dilution", and "conc"—along with any custom columns from the uploaded template.

The app rounds analyte concentrations to three significant figures for easier interpretation, especially when sample values vary widely or are measured at different dilutions. For example, a high sample measured at 12345.678 ng/ml and a low sample at 1.23456 ng/ml would appear as 12300 and 1.23, respectively.

Users can modify the number of significant digits by adjusting the parameter in the "Rounding Options" section in the sidebar. Additionally, selecting "Round" as the rounding function allows values to be rounded to a specific decimal place.

Users can export the table with calculated concentrations as an Excel or CSV file by clicking the “Download results” button.

Result Summary

In the "Result Summary" tab, users can aggregate results based on common values found in the following descriptive columns:

- sample.names

- sample.date
- dilution

Any additional columns provided by the user can also be used for further aggregation. For each combination of the selected parameters, the app calculates:

- Number of samples in the group (“n” column).
- Mean analyte concentration ("mean" column).
- Minimum and maximum observed concentrations (“min” and “max” columns).

The aggregated results can be exported as an Excel or CSV file for further analysis.

Report

The "Report" tab provides an option to generate a detailed summary of the experiment. Users need to fill in only one field – "Title" – the experiment name. By clicking the “Download Report” button, the app generates a Word (.docx) file containing:

- The current system date and experiment name.
- Plots: Standard curve and residuals vs. fitted values.
- A table of regression coefficients and the type of linear model used.
- The Results Table and Summary Table.

This report file can be further edited and customized by users as needed.

Sample Data Sets

The Linearity App includes three sample datasets, generated from real laboratory experiments, which users can reference and process within the app.

BCA assay

File: #01_BCA_assay_semicolon-delimited.csv

This dataset contains results from a bicinchoninic acid (BCA) assay, used to quantify protein concentration. The experiment tested four purified recombinant protein samples from four transfection experiments (T1–T4), each measured at two dilution factors (5 and 10).

Experimental Details:

- Each sample was tested in triplicate at each dilution.
- Two BSA titrations with known concentrations (2 mg/mL to 0.031 mg/mL) were used to generate the standard curve.
- The data is provided in CSV format, using a semicolon (;) as the delimiter.

Sandwich ELISA

File: #02_Sandwich ELISA (sMICB)_comma-delimited.csv

This dataset originates from a sandwich ELISA experiment, measuring soluble MICA (sMICB) levels in blood plasma samples from prostate cancer patients.

Experimental Details:

- Plasma samples were serially diluted two-fold before testing.
- The assay was standardized using the recombinant extracellular domain of MICB*005, titrated twice from 5 ng/mL to 0.078 ng/mL.
- The data is provided in CSV format, using a comma (,) as the delimiter.

Greiss assay

File: #03_Griess_assay_Excel.xlsx

This dataset contains results from a Griess assay, which measures nitrite production as an indicator of immune cell polarization. The experiment evaluated nitrite levels in the media of macrophages derived from mouse bone marrow, categorized into three polarization states: M0 (non-polarized), M1 (classically polarized), or M2 (alternatively polarized).

Experimental Details:

- Samples were collected on two separate days and tested in duplicate.
- Two nitrite titrations (from 100 µM to 1.56 µM) in cell culture medium were used for assay standardization.
- The data is provided in an Excel (.xlsx) file.